

A New Mode of Ca^{2+} Signaling by G Protein-Coupled Receptors: Gating of IP_3 Receptor Ca^{2+} Release Channels by $\text{G}\beta\gamma$

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Summary

The most common form of Ca^{2+} signaling by Gq-coupled receptors entails activation of $\text{PLC}\beta 2$ by $\text{G}\alpha\text{q}$ to generate IP_3 and evoke Ca^{2+} release from the ER [1]. Another form of Ca^{2+} signaling by G protein-coupled receptors involves activation of Gi to release $\text{G}\beta\gamma$, which activates $\text{PLC}\beta 1$ [2]. Whether $\text{G}\beta\gamma$ has additional roles in Ca^{2+} signaling is unknown. Introduction of $\text{G}\beta\gamma$ into cells activated Ca^{2+} release from the IP_3 Ca^{2+} pool and Ca^{2+} oscillations [3, 4]. This can be due to activation of $\text{PLC}\beta 1$ or direct activation of the IP_3 R by $\text{G}\beta\gamma$. We report here that $\text{G}\beta\gamma$ potentially activates the IP_3 receptor. Thus, $\text{G}\beta\gamma$ -triggered $[\text{Ca}^{2+}]_i$ oscillations are not affected by inhibition of $\text{PLC}\beta$. Coimmunoprecipitation and competition experiments with $\text{G}\beta\gamma$ scavengers suggest binding of $\text{G}\beta\gamma$ to IP_3 receptors. Furthermore, $\text{G}\beta\gamma$ inhibited IP_3 binding to IP_3 receptors. Notably, $\text{G}\beta\gamma$ activated single IP_3 R channels in native ER as effectively as IP_3 . The physiological significance of this form of signaling is demonstrated by the reciprocal sensitivity of Ca^{2+} signals evoked by Gi - and Gq -coupled receptors to $\text{G}\beta\gamma$ scavenging and $\text{PLC}\beta$ inhibition. We propose that gating of IP_3 R by $\text{G}\beta\gamma$ is a new mode of Ca^{2+} signaling with particular significance for Gi -coupled receptors.

Results and Discussion

$\text{G}\beta\gamma$ -Evoked Ca^{2+} Release Is Independent of PLC Activation

In a previous work we reported that infusion of $\text{G}\beta\gamma$ into pancreatic acinar cells evoked two types of $[\text{Ca}^{2+}]_i$ oscillations: high-frequency, short-lasting spikes and low-frequency, longer-lasting Ca^{2+} spikes (see also Figures 1G and 1H). These effects were blocked by two $\text{G}\beta\gamma$ scavengers, the $\text{G}\beta\gamma$ binding domain of the β -adrenergic receptor kinase 1 ($\beta\text{ARK}1$) and a mutant $\text{G}\alpha i$ (G203A), as well as by heparin, a competitive inhibitor of the IP_3 R [4]. These findings were attributed to activation of $\text{PLC}\beta 1$

by $\text{G}\beta\gamma$. However, the present findings refute this interpretation. In pancreatic acini, one can reliably estimate $[\text{Ca}^{2+}]_i$ by measuring the Ca^{2+} -activated Cl^- current, which reflects Ca^{2+} spiking at the apical pole [5]. With such measurements, two independent protocols indicate that $\text{G}\beta\gamma$ activated Ca^{2+} release independent of $\text{PLC}\beta$ activation. U73122, an inhibitor of all $\text{PLC}\beta$ isoforms, abolished $[\text{Ca}^{2+}]_i$ oscillations evoked by stimulation of the Gq-coupled M3 receptor with a low concentration of carbachol, and it largely inhibited the sustained response evoked by 1 mM carbachol (Figure 1A). We could still induce maximal Ca^{2+} release in the presence of U73122 by using the Ca^{2+} ionophore ionomycin. By contrast, U73122 failed to inhibit $[\text{Ca}^{2+}]_i$ oscillations induced by $\text{G}\beta\gamma$ (Figures 1B and 1C). $\text{G}\beta\gamma$ -induced $[\text{Ca}^{2+}]_i$ oscillations were observed in 16 out of 19 control cells and, similarly, in 5 out of 6 cells treated with U73122. Comparable results were obtained with an anti-PIP2 antibody, another inhibitor of $\text{PLC}\beta$ -mediated Ca^{2+} signaling. This anti-PIP2 antibody has been demonstrated to specifically inhibit several PIP2-dependent actions [6]. Because antibody diffusion into cells from a patch pipette is slow, its effects were expected to be time dependent, as observed (Figures 1D and 1E). After 200 and 400 s of infusion into the cells, the responses to maximal stimulation of Gq-coupled M3 and bombesin receptors were blocked by about 60% and close to 95%, respectively (Figure 1F). Nevertheless, the anti-PIP2 antibody failed to block the responses to $\text{G}\beta\gamma$, even after 20 min of infusion, while inhibiting the responses to carbachol in the same cells (Figures 1G and 1H). A similar lack of effects of anti-PIP2 antibody on the responses to $\text{G}\beta\gamma$ were observed in 6 out of 8 cells.

The results in Figure 1, showing that inhibition of PLC activity by two different inhibitors is without effect on $\text{G}\beta\gamma$ -activated Ca^{2+} signaling, suggest that $\text{G}\beta\gamma$ can activate Ca^{2+} release from internal stores independently of IP_3 production. Below we show that this is due to activation of the IP_3 R Ca^{2+} release channel by $\text{G}\beta\gamma$.

$\text{G}\beta\gamma$ Interacts with IP_3 Receptors

Interaction between $\text{G}\beta\gamma$ and IP_3 Rs was examined by coimmunoprecipitation (co-IP) with cerebellar extracts, a rich source of IP_3 R1 and $\text{G}\beta\gamma$. The low abundance of IP_3 Rs precluded comparable experiments in pancreatic acini, in particular the IP_3 binding assays. Cerebellar extracts were fractionated on a linear sucrose gradient to isolate fractions enriched in both proteins (Figure 2A, fractions 8–11). In reciprocal IP, both proteins were found to interact (Figures 2B and 2C). Notably, the $\text{G}\beta\gamma$ scavengers βARK and $\text{G}\alpha i$ (G203A) prevented the co-IP. Similarly, a peptide (βARKP) that constitutes the $\text{G}\beta\gamma$ binding motif of βARK and inhibits binding of $\text{G}\beta\gamma$ to target proteins [7, 8] prevented the co-IP. These findings are consistent with dissociation of the IP_3 R1- $\text{G}\beta\gamma$ complex by these agents. The significance of these findings is that (1) the interaction of $\text{G}\beta\gamma$ with the IP_3 R1 is specific and (2) the binding of $\text{G}\beta\gamma$ to IP_3 R1 is not tight because

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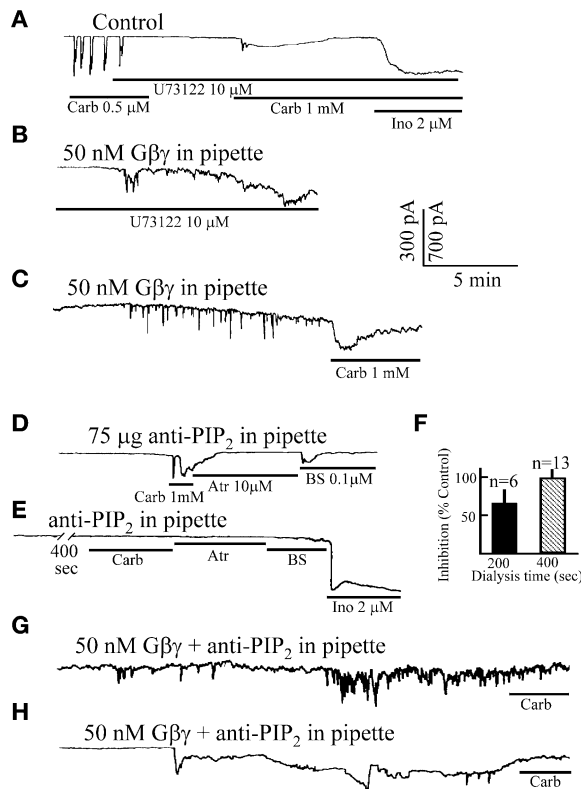


Figure 1. Gβγ-Activated [Ca²⁺]_i Oscillations Are Independent of PLCβ Activity

Whole-cell Ca²⁺-activated Cl⁻ current was used for following changes in [Ca²⁺]_i. Rat pancreatic acinar cells were infused with a control pipette solution (A), pipette solution containing 50 nM Gβγ (B, C, G, H), or pipette solution containing 75 μg/ml anti-PIP₂ antibody (D–F). In (A) and (B), the cells were treated with 10 μM U73122, as indicated. Scale bars indicate the period of exposure to 0.5 μM or 1 mM carbachol (carb), 0.1 μM bombesin (BS), 2 μM ionomycin (Ino), or 10 μM atropine (Atr). In all experiments, recording commenced about 15 s after the whole-cell configuration was established.

it can be competed away by two proteins that bind Gβγ and it was disrupted by a peptide that interferes with binding of Gβγ to target proteins.

The majority of Gβγ was retained at the top of the gradient, whereas most of the IP₃R1 migrated to the bottom (Figure 2A). The separation of IP₃R1 from Gβγ allowed us to study the effects of Gβγ on a vital IP₃R function, that of IP₃ binding. IP₃ binding to fraction 4 (containing only Gβγ) was used as a control. IP₃ binding to IP₃R1 in fraction 10, which contains both IP₃R and Gβγ, was low (Figure 2D). Notably, binding was increased about 5-fold by the Gβγ binding inhibitor βARKP (Figure 2D) in a concentration-dependent manner (Figure 2E), as well as by the Gβγ scavenger βARK. By contrast, Gβγ had no further effect on IP₃ binding. Reciprocal effects on IP₃ binding to the IP₃R1 were observed in fraction 15, which contained IP₃R but lacked Gβγ. Thus, βARKP and βARK had no effect on IP₃ binding, whereas Gβγ inhibited it in a concentration-dependent manner. These findings suggest that Gβγ modulates IP₃ binding to the IP₃ receptors. To determine the

state of the native IP₃Rs with respect to Gβγ binding, we measured the effects of βARKP and Gβγ on IP₃ binding to IP₃Rs in cerebellar microsomes. Figure 2E shows that βARKP had no effect, whereas Gβγ inhibited IP₃ binding, suggesting that most native IP₃Rs are not associated with Gβγ.

Gβγ Activates the IP₃ Receptors Ca²⁺ Release Channel

Gβγ could inhibit IP₃ binding to IP₃Rs by allosteric modification of the IP₃ binding site or by competing with IP₃ for binding to the same site/region of the IP₃R1. To study the significance of this modulation, we examined the effects of purified recombinant Gβγ on the single-channel activity of native IP₃ receptors recorded in the nuclear envelope of *Xenopus* oocytes. Normally, channel gating is not observed unless the patch pipette contains IP₃ (Figure 3). Remarkably, Gβγ activated channel gating in the absence of added IP₃. Gβγ elicited channel activity as efficaciously as IP₃ (Figure 3C) and to comparable levels of channel-open probability (Figure 3F). The addition of Gαi(G203A) to scavenge Gβγ eliminated its ability to activate channel gating (Figure 3E), demonstrating the specificity of its effect. Furthermore, channels activated by Gβγ were inhibited by heparin (Figure 3D), a competitive IP₃R channel inhibitor [4]. Electrophysiological demonstration that Gβγ activates channel gating, together with biochemical evidence that Gβγ inhibits IP₃ binding, indicates that Gβγ interacts with IP₃Rs and stabilizes a channel conformation that exhibits activity comparable to that of the IP₃ bound channel. These results therefore suggest that IP₃ and Gβγ binding represent two alternative modes of activating IP₃ receptors.

Ca²⁺ signaling evoked by Gq-coupled receptors is dependent on αq-mediated activation of PLCβ, whereas Ca²⁺ signaling evoked by Gi-coupled receptors is mediated by Gβγ [9]. Therefore, to examine the physiological significance of Gβγ gating of IP₃ channels, we compared the effects of inhibiting PLCβ or scavenging Gβγ on Ca²⁺ signaling evoked by stimulation of Gq- and Gi-coupled receptors in the same cells with carbachol and vasoactive intestinal peptide (VIP), respectively. Activation of the Gs-coupled VIP receptors was shown to evoke Ca²⁺ spiking in pancreatic acini that can be mimicked by an increase in cytoplasmic cAMP [10]. In subsequent work we showed that although the M3 receptors activate Gαq [4], the VIP receptors use a Gs/Gi switching mechanism [11] to activate Gi and promote the release of Gβγ that is essential for Ca²⁺ signaling by these receptors [12]. For these experiments, we employed U73122 to inhibit PLCβ so that we could compare the effects of VIP on IP₃ production and Ca²⁺ signaling. Inhibition of PLCβ with U73122 (0.2 or 0.5 μM) inhibited or reduced the Ca²⁺ signaling response to 0.5 μM or 100 μM carbachol, respectively, as expected, whereas it had minimal effects on the Ca²⁺ signals evoked by VIP (Figures 4A and 4B). U73122 similarly inhibited the carbachol response in cells that had not been treated with VIP or exposed to U73122 for a long period of time (Figure 4C). Reciprocal observations were made in cells infused with as little as 20 nM of the Gβγ scavenger Gαi (G203A), which abolished the response to VIP without affecting

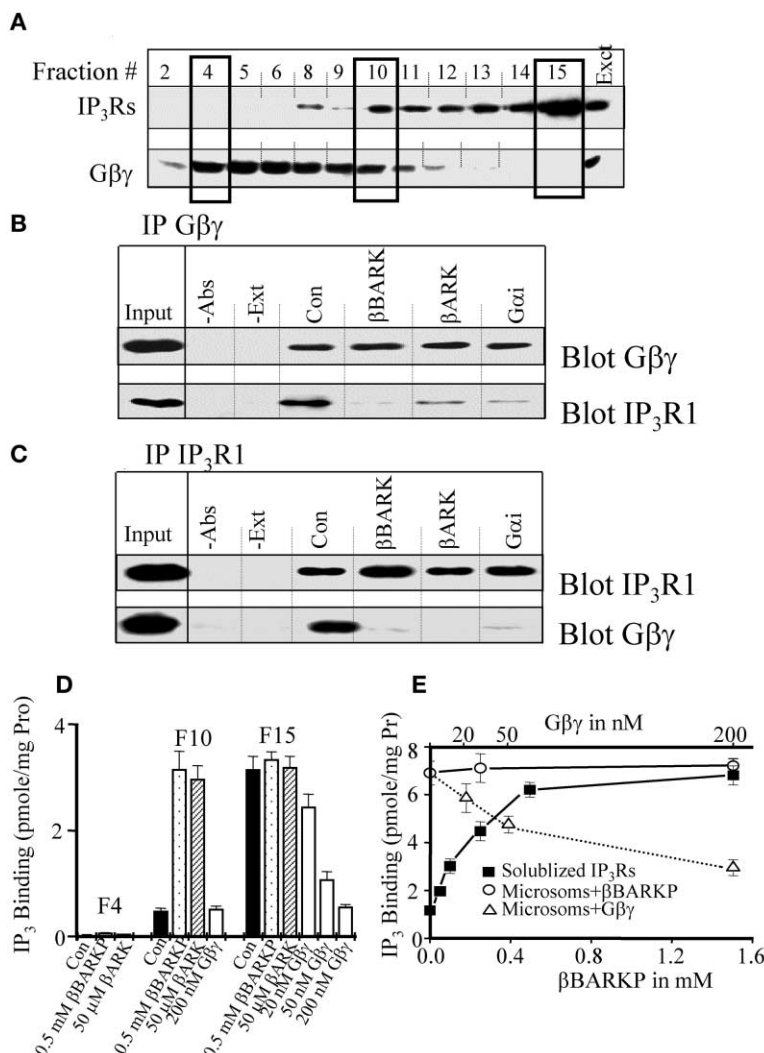


Figure 2. Co-IP of IP₃R1 and Gβγ and Effect of Gβγ on IP₃ Binding to IP₃R1

(A) Blot of IP₃R1 and Gβγ in 15 fractions collected from a linear sucrose gradient. Fractions similar to 8–11 were pooled and used for immunoprecipitating Gβγ (B) or IP₃R1 (C). The pooled extracts were incubated with buffer (Control, Con); 0.5 mM βARKP, 50 μM βARK, or 200 nM Gαi(G203A) (Gαi) for 30 min at 0°C before addition of antibodies and beads. For controls, in the first lane in each case the primary antibodies were omitted (–Abs), and in the second lane the extract was omitted (–Ext). The input represents 13% of the material used for the IP. Fractions 4, 10, and 15 were used for the IP₃ binding assay in panel (D). The extracts from each fraction were added to incubation medium containing the indicated final concentrations of Gβγ, βARKP, and βARK and incubated for 30 min at 0°C before addition of ³H-IP₃ and a further incubation for 15 min at 0°C. At the end of the incubations, the solubilized IP₃R1 was precipitated with ethylene glycol, and binding of IP₃ was determined. Similar experiments were performed with fraction 10 from a separate gradient and [βARKP] between 0.05 and 1.5 mM. The dose response for βARKP-mediated increase in IP₃ binding is plotted in panel E (squares). Intact cerebellar microsomes were used to test the effect of βARKP (circles) and Gβγ (triangles) on IP₃ binding to IP₃R1 in native membranes. The behavior of IP₃R1 in cerebellar microsomes was similar to that in fraction 15, indicating that the IP₃R1 in isolated cerebellar microsomes is largely free of Gβγ and that Gβγ can regulate the IP₃R1 in native membranes.

the responses to either low or high concentrations of carbachol (Figure 4D). It was necessary to increase the concentration of Gαi (G203A) to 50 (Figure 4E) and 200 nM (Figure 4F) to inhibit the responses to 0.5 μM and 100 μM carbachol, respectively. These findings were corroborated by measurements of IP₃ production in cells stimulated by the two agonists. VIP failed to elicit a measurable increase in IP₃, in agreement with findings with other native Gi-coupled receptors (Figure 4G), whereas 0.5 μM carbachol elicited a readily measurable increase that was inhibited by 0.5 μM U73122. The same concentration of U73122 also partially inhibited IP₃ production in response to maximal stimulation with 100 μM carbachol. Taken together, these results highlight the two modes of signal transduction by GPCR and their differential dependence on IP₃ production and Gβγ. These results provide physiological support for the idea that Ca²⁺ signals elicited by Gi-coupled receptors, although mediated by Ca²⁺ release through the IP₃Rs, are promoted by IP₃R activation by Gβγ.

The combined findings of the present work indicate that Gβγ activates IP₃Rs. The IP₃R is the third channel known to be regulated by Gβγ, the others being the

inward-rectifying K⁺ channels and the L-type Ca²⁺ channels [13]. Gβγ regulates the K⁺ and Ca²⁺ channels by directly binding to these channels [13]. The observed co-IP of Gβγ and IP₃R and inhibition of IP₃ binding to solubilized IP₃Rs by Gβγ are consistent with such direct regulation.

The present work expands the modes by which G proteins can activate Ca²⁺ signaling. Thus, GPCRs can activate Ca²⁺ signaling by activation of PLCβ to generate IP₃ or by activation of IP₃Rs with Gβγ. Inhibition of all forms of Ca²⁺ signaling by the Gβγ scavengers and inhibitors (Figure 4 and [4]) suggests that Gβγ participates in both Gq- and Gi-dependent Ca²⁺ signaling. However, activation of IP₃Rs by Gβγ appears to be of particular importance for Gi-coupled receptors. Activation of these receptors releases Gαi and Gβγ. Although it is generally assumed that Gβγ activates PLCβ1 to trigger a Ca²⁺ signal [9], it is noteworthy that it is difficult to detect any IP₃ production by activation of Gi-coupled receptors, as illustrated here for the VIP receptor (Figure 4). Our results suggest that an alternative explanation is that Gβγ directly activates IP₃Rs, independently of IP₃ production. Indeed, the Ca²⁺ signals evoked by VIP

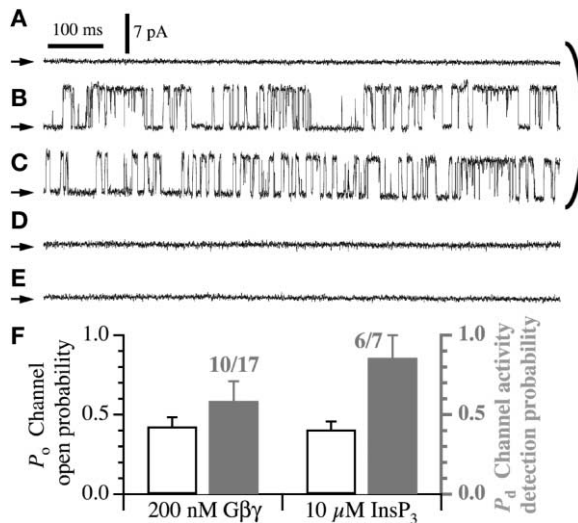


Figure 3. IP₃-Independent Gating of IP₃R1 by Gβγ

Typical patch-clamp current traces from outer membrane patches obtained from isolated *Xenopus* oocyte nuclei. Arrows indicate the closed-channel current level. (A) The pipette solution contained 0.02% lubrol; no channel activity was observed in any of 18 patches. (B) The pipette solution contained 200 nM Gβγ and 0.02% lubrol; InsP₃R channel activities were observed in ten of 17 patches. (C) The pipette solution contained 10 μM InsP₃; InsP₃R channel activities were observed in six of seven patches. Current traces (A)–(C) (enclosed with the brace) were recorded in membrane patches obtained from the same region of the same oocyte nuclei. (D) Pipette solution contained 200 nM Gβγ, 100 mg/ml heparin, and 0.02% lubrol; no channel activity was observed in any of 12 patches. In the same region of the same oocyte nuclei, InsP₃R channel activities were observed in eight of 11 patches with pipette solution containing 10 μM InsP₃. (E) Pipette solution contained 200 nM Gβγ, 500 nM Gαi(G203A), and 0.02% lubrol; no channel activity was observed in any of five patches. In the same region of the same oocyte nuclei, InsP₃R channel activities were observed in nine of ten patches with pipette solution containing 10 μM InsP₃. (F) Channel-open probability (P_o, open bars) and channel activity detection probability (P_d = number of membrane patches exhibiting InsP₃R channel activities/total number of membrane patches obtained, filled bars) for pipette solutions containing 200 nM Gβγ and 0.02% lubrol, or 10 μM InsP₃. P_o and P_d in the presence of the two agonists exhibit no statistically significant differences ($p > 0.05$).

stimulation were exquisitely sensitive to scavenging Gβγ with Gαi (G203A), and at the same time they were not inhibited by concentrations of the PLCβ inhibitor U73122 that inhibited a more intense signals evoked by the Gq-coupled M3 receptor in the same cells. Hence, we propose that a major mechanism to evoke Ca²⁺ signaling by Gi-coupled receptors is direct activation of IP₃Rs by Gβγ.

Activation of PLCβ and IP₃Rs by Gβγ is not mutually exclusive. Gβγ appears to activate the two proteins with similar potency. Half maximal activation of all PLCβ isoforms requires 100 nM or higher Gβγ [9]. This value is in the same range as the concentration that markedly activated the IP₃R (200 nM Gβγ). This would suggest that the mode of organization of the signaling complex will determine the preferential pathway used by the Gi-coupled receptors to activate Ca²⁺ signaling. Alternatively, the two mechanisms may be used in series to initiate a Ca²⁺ signal: local activation of IP₃Rs with Gβγ

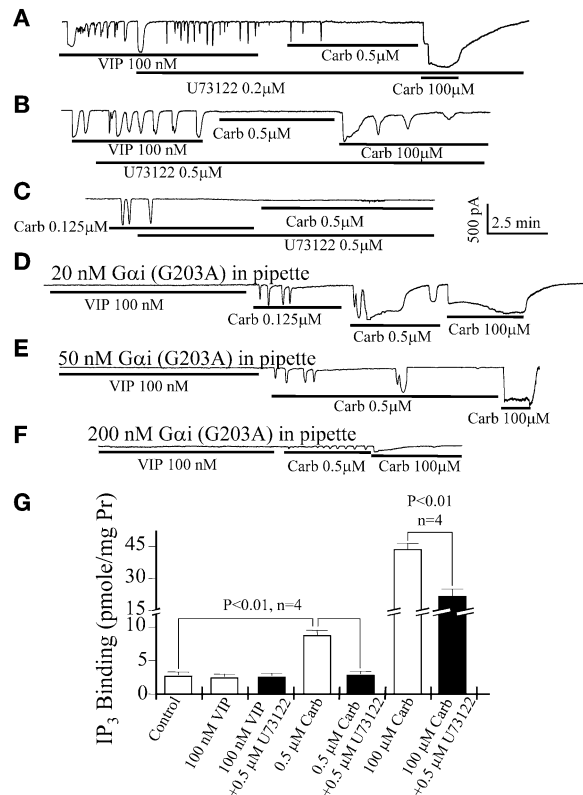


Figure 4. Differential Sensitivity of Gi- and Gq-Dependent Ca²⁺ Signaling to Inhibition of PLCβ and Gβγ

Mouse pancreatic acini were dialyzed with control pipette solution (A–C) or pipette solution containing 20 nM (D), 50 nM (E), or 200 nM (F) Gαi(G203A) for 7 min prior to stimulation with the indicated concentrations of VIP and carbachol as indicated by the bars. In (A), after the control period for recording the response to VIP, the cells were exposed to 0.2 μM U73122 for the remainder of the experiment. In (B), the cells were exposed to 0.5 μM U73122, and in panel (C) oscillations similar to those evoked by VIP were induced with 0.125 μM carbachol before the exposure to 0.5 μM U73122. Note that in mouse pancreatic acini 0.5 μM carbachol generated a robust Ca²⁺ signal (D). Similar results to those in panels (A)–(F) were obtained in at least three experiments. Panel (G) shows results of two experiments performed in duplicates to measure IP₃ production by cells stimulated with the indicated concentrations of agonist. A portion of the cells was treated with 0.5 μM U73122 for 2 min (filled columns) prior to stimulation with agonists. The results are the mean ± S.E.M of the four determinations.

and a more distal activation of Ca²⁺ release by IP₃. Such a mechanism may also contribute to Ca²⁺ signaling by Gq-coupled receptors. Whereas IP₃ can diffuse rapidly in the cytosol [14], diffusion of Gβγ is very limited [3]. Therefore, Gβγ would be ideal for local activation of IP₃Rs most adjacent to the plasma membrane. Cell signaling, including Ca²⁺ signaling [15], occurs by the assembly of protein complexes in cellular microdomains, such as caveolae [16], the post-synaptic densities [17], and the luminal pole of epithelial cells [15]. In the case of Ca²⁺ signaling, the complexes include receptors, G proteins, IP₃Rs, and Ca²⁺ pumps [15], and they are the sites from which Ca²⁺ waves initiate near the plasma membrane [5, 18–20]. Receptor-triggered release of Gβγ within these complexes could initiate and/or facilitate

activation of the sub-set of IP₃Rs that function as a trigger pool to launch $[Ca^{2+}]_i$ oscillations and Ca^{2+} waves.

In summary, the present work provides evidence for IP₃-independent regulation of IP₃Rs by Gβγ. The widespread expression of G proteins and GPCRs suggests that this alternative mode of activation of the IP₃Rs is a general mechanism present in all cells. Physiologically, it appears that activation of the IP₃ receptors by Gβγ is central to Gi-coupled receptor-generated Ca^{2+} signals.

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References

- Berridge, M.J. (1993). Inositol trisphosphate and calcium signaling. *Nature* 367, 315–325.
- Pierce, K.L., Premont, R.T., and Lefkowitz, R.J. (2002). Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.* 3, 639–650.
- Stehno-Bittel, L., Krapivinsky, G., Krapivinsky, L., Perez-Terzic, C., and Clapham, D.E. (1995). The G protein βγ subunit transduces the muscarinic receptor signal for Ca^{2+} release in *Xenopus* oocytes. *J. Biol. Chem.* 270, 30068–30074.
- Zeng, W., Xu, X., and Muallem, S. (1996). Gβγ transduces $[Ca^{2+}]_i$ oscillations and Gαq a sustained response during stimulation of pancreatic acinar cells with $[Ca^{2+}]_i$ -mobilizing agonists. *J. Biol. Chem.* 271, 18520–18526.
- Thorn, P., Lawrie, A.M., Smith, P.M., Gallacher, D.V., and Petersen, O.H. (1993). Local and global cytosolic Ca^{2+} oscillations in exocrine cells evoked by agonists and inositol trisphosphate. *Cell* 74, 661–668.
- Hilgemann, D.W., Feng, S., and Nasuhoglu, C. (2001). The complex and intriguing lives of PIP₂ with ion channels and transporters. <http://stke.sciencemag.org/cgi/reprint/sigtrans;2001/111/re19>.
- Koch, W.J., Hawes, B.E., Inglese, J., Luttrell, L.M., and Lefkowitz, R.J. (1994). Cellular expression of the carboxyl terminus of a G protein-coupled receptor kinase attenuates G beta gamma-mediated signaling. *J. Biol. Chem.* 269, 6193–6197.
- Colombo, M.I., Inglese, J., D'Souza-Schorey, C., Beron, W., and Stahl, P.D. (1995). Heterotrimeric G proteins interact with the small GTPase ARF. Possibilities for the regulation of vesicular traffic. *J. Biol. Chem.* 270, 24564–24571.
- Rhee, S.G. (2001). Regulation of phosphoinositide-specific phospholipase C. *Annu. Rev. Biochem.* 70, 281–312.
- Kase, H., Wakui, M., and Petersen, O.H. (1991). Stimulatory and inhibitory actions of VIP and cAMP on cytoplasmic Ca^{2+} signal generation in pancreatic acinar cells. *Pflugers Arch.* 419, 668–670.
- Daaka, Y., Luttrell, L.M., and Lefkowitz, R.J. (1997). Switching of the coupling of the β₂-adrenergic receptor to different G proteins by protein kinase A. *Nature* 390, 88–91.
- Luo, X., Zeng, W., Xu, X., Popov, S., Davignon, I., Wilkie, T.M., Mumby, S.M., and Muallem, S. (1999). Alternate coupling of receptors to Gs and Gi in pancreatic and submandibular gland cells. *J. Biol. Chem.* 274, 17684–17690.
- Dascal, N. (2001). Ion-channel regulation by G proteins. *Trends Endocrinol. Metab.* 12, 391–398.
- Allbritton, N.L., Meyer, T., and Stryer, L. (1992). Range of messenger action of calcium ion and inositol 1,4,5-trisphosphate. *Science* 258, 1812–1815.
- Kiselyov, K., Shin, D.M., and Muallem, S. (2003). Signaling specificity in GPCR-dependent Ca^{2+} signaling. *Cell. Signal.* 15, 243–253.
- Anderson, R.G., and Jacobson, K. (2002). A role for lipid shells in targeting proteins to caveolae, rafts, and other lipid domains. *Science* 296, 1821–1825.
- Hering, H., and Sheng, M. (2001). Dendritic spines: structure, dynamics and regulation. *Nat. Rev. Neurosci.* 12, 880–888.
- Kasai, H., Li, Y.X., and Miyashita, Y. (1993). Subcellular distribution of Ca^{2+} release channels underlying Ca^{2+} waves and oscillations in exocrine pancreas. *Cell* 74, 669–677.
- Xu, X., Zeng, W., Diaz, J., and Muallem, S. (1996). Spatial compartmentalization of Ca^{2+} signaling complexes in pancreatic acini. *J. Biol. Chem.* 271, 24684–24690.
- Straub, S.V., Giovannucci, D.R., and Yule, D.I. (2000). Calcium wave propagation in pancreatic acinar cells: functional interaction of inositol 1,4,5-trisphosphate receptors, ryanodine receptors, and mitochondria. *J. Gen. Physiol.* 116, 547–560.